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Freezing and air drying determine phytochemical traits and antioxidant potential of selected edible flowers

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ABSTRACT

This study investigated the phytochemical composition and antioxidant potential of edible flower according A factorial experiment on six species (*Rosa* spp., *Calendula officinalis*, *Viola odorata*, *Trifolium pratense*, *Sambucus nigra*, and *Borago officinalis*) using two solvents (aqueous and ethanolic) and four sample states (fresh, freeze-dried, and air-dried at 30 and 50 °C) a. Bioactive compounds were evaluated by total phenolic content (TPC) and monomeric anthocyanin content (MAC), while antioxidant activities were assessed through DPPH, ABTS, FRAP, and reducing power (RP) assays. Results showed that both main factors and their interactions significantly influenced phytochemicals and antioxidant capacity. Fresh and freeze-dried flowers retained the highest levels of phenolics, anthocyanins, and antioxidant activity, whereas hot-air drying at 50 °C caused the greatest losses. Ethanolic extraction consistently outperformed aqueous extraction in recovering bioactive compounds and antioxidant potential. Correlation analysis indicated moderate positive associations between TPC and antioxidant assays, and strong correlations between MAC and RP. The study was limited to six species, selected drying temperatures, and two solvent types. Nevertheless, findings highlight that freeze-drying combined with ethanolic extraction is the most effective method to preserve phytochemical integrity and antioxidant capacity of edible flowers. These insights can guide food and nutraceutical industries in optimizing processing strategies for maintaining the nutritional and functional properties of floral ingredients.

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1. Introduction

Edible flowers have garnered significant attention in recent years due to their rich phytochemical profiles and potential health benefits, positioning them as promising ingredients in the modern food industry (López *et al*., 2023). Species such as *Viola odorata*, *Trifolium pratense*, *Sambucus nigra*, *Borago officinalis*, *Rosa* spp. (edible rose), and *Calendula officinalis***,** each with a distinct genetic profile, are among those recognized for their diverse bioactive compounds, including polyphenols, flavonoids, and carotenoids. These compounds are associated with potent antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable in functional foods and nutraceuticals (Selvi *et al*., 2020; Pires *et al*., 2023).

The preservation of these thermolabile bioactive compounds is crucial for maintaining the health benefits and commercial value of edible flowers. Drying is the most common method employed to extend shelf life and enable year-round availability; however, the chosen technique can significantly impact the retention of phytochemicals. Freeze-drying (lyophilization) is often considered the gold standard, as it effectively preserves bioactive compounds by sublimating water under vacuum, maintaining the structural integrity of plant matrices, and minimizing thermal degradation (López *et al*., 2023). In contrast, conventional hot-air drying methods, depending on temperature and duration, may lead to substantial losses of sensitive compounds through oxidative and enzymatic reactions (Stefaniak and Grzeszczuk, 2020; Guiné *et al*., 2021).

Recent studies have highlighted the critical importance of optimizing drying conditions to enhance the retention of bioactive compounds in various edible flowers. For instance, a study by Zawiślak *et al.* (2022) demonstrated that freeze-drying preserved higher levels of polyphenols and anthocyanins in *Trifolium pratense*, *Viola odorata*, and *Sambucus nigra* compared to air-drying at 30°C and 50°C. Similarly, research on *Calendula officinalis* has shown that freeze-drying outperforms convective drying in preserving antioxidant capacity (Tyskiewicz *et al*., 2022). The species-specific response to drying is a key factor, as the matrix composition and compound stability vary greatly among flowers (Touvron *et al*., 2023).

In the context of northern Iran, where the soils are predominantly heavy to very heavy Luvisols derived from loess deposits, the cultivation of these edible flower species presents an opportunity to explore their unique phytochemical profiles-which are known to be influenced by such edaphic factors (Mishra *et al*., 2022)-and their underlying genetic makeup, and the effects of various drying techniques on their preservation. Understanding the complex interplay between cultivation conditions (e.g., soil, climate), genetic background, species-specific characteristics, postharvest processing, and the retention of bioactive compounds is essential for maximizing the health benefits of these flowers and successfully integrating them into functional food products (Fernandes *et al*., 2023).

The aim of this study was to investigate the effects of different drying techniques (freeze-drying and air-drying at various temperatures) on the phytochemical composition (total phenolics, flavonoids, anthocyanins) and antioxidant activity of six edible flower species: *Viola odorata*, *Trifolium pratense*, *Sambucus nigra* (elderberry), *Borago officinalis* (borage), *Rosa* spp. (edible rose, cultivar ‘Himalayan Mystery’), and *Calendula officinalis* (pot marigold). In addition, the study sought to evaluate the influence of species-specific characteristics on the stability of their bioactive compounds after drying. The findings aim to provide practical, evidence-based insights for optimizing postharvest processing, particularly drying methods, to preserve health-promoting compounds and enhance the functional potential of edible flowers in food and nutraceutical applications.

2. MATERIALS AND METHODS

2.1. Material Preparation

Flowers of six species-*Viola odorata* L. (sweet violet), *Trifolium pratense* L. (red clover), *Sambucus nigra* L. (elderberry), *Borago officinalis* L. (borage), *Rosa* spp. (cultivar 'Himalayan Mystery', edible rose), and *Calendula officinalis* L. (pot marigold)-were hand-harvested at full bloom during the spring and early summer of 2025 from the *Camellia* plant and flower production site in northern Iran. The collected material was subsequently transferred to the Agricultural Laboratories of Azad University, located in Chalus and Rasht, for further analysis. Soils in the collection area, derived mainly from loess deposits, are classified as heavy to very heavy Luvisols (Esfandiarpour-Boroujeni *et al*., 2018; Roozitalab *et al*., 2018; Owliaie, 2023).

For *Rosa* spp., only petals were separated from the hypanthium and reproductive structures to avoid bitterness. All other inflorescences were carefully cleaned to remove extraneous material, and preliminary washing was avoided to prevent leaching of water-soluble compounds (Grzegorczyk *et al*., 2021). Each species was then divided into four representative portions: one portion was immediately analyzed in its fresh state, while the remaining three portions were subjected to different drying protocols to assess the impact of drying methods on phytochemical preservation.

The collected material for each species was divided into four representative parts. One part was immediately analyzed in its fresh state. The remaining three parts were subjected to different drying protocols to evaluate the impact of drying techniques on phytochemical preservation:

Freeze-drying (Lyophilization): Using a Labor, MIM OE 950 lyophilizer (Budapest, Hungary). This method is considered the benchmark for preserving thermolabile compounds (Calín-Sánchez *et al.*, 2020).

Air-drying at 30 °C**:** For 5 h in a Binder ED series oven (Tuttlingen, Germany).

Air-drying at 50 °C**:** For 3 h in a Binder ED series oven (Tuttlingen, Germany).

The dry matter content for both fresh and dried samples was determined gravimetrically by drying in an oven at 105 **°C** until a constant weight was achieved, according to the standard AOAC method (Thiex *et al*., 2012).

2.1.1. Preparation of Ethanolic Extracts

Ethanolic extracts were prepared using a standardized homogenization-assisted extraction method, adapted for efficient polyphenol recovery from floral tissues (Yammine et al., 2021). Briefly, 5 g of fresh flower material (or petals for rose) was homogenized with 95 mL of a hydroethanolic solution (Ethanol: 2% HCl; 95:5, v/v) at 25 °C for 5 min. The homogenate was then stirred magnetically for 45 min to complete the extraction. Subsequently, the mixture was centrifuged at 5500 rpm (relative centrifugal force ~4000 × g) for 10 min. The resulting supernatant was decanted to obtain a 5% (w/v) ethanolic extract. For the dried flower samples, an equivalent amount, recalculated based on the previously determined dry matter content to match the dry weight of 5 g of fresh material, was subjected to the identical extraction procedure. This ensured the preparation of 5% extracts directly comparable to those from the fresh raw material.

2.1.2. Preparation of Infusions

Infusions were prepared to simulate a traditional preparation method (Fernández et al., 2021). Exactly 5g of fresh flower material was infused with 95 mL of boiling distilled water (100 °C) under continuous stirring for 5 min. The infusion was then filtered through filter paper and allowed to cool to room temperature. For dried samples, the weight of the plant material was similarly adjusted based on its dry matter content to yield a 5% infusion equivalent to that from fresh material.

2.2. Analytical Methods

All experiments were conducted in triplicate (n = 3), and data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's HSD test to determine significant differences (P < 0.05) between means. Statistical analyses were performed using SPSS.

Table 1. Treatment used in experiment.

Treatment Type	Treatment	Code
Plant species	Rosa spp. (edible rose)	R
	Calendula officinalis L.	C
	Viola odorata	V
	Trifolium pratense L.	T
	Sambucus nigra L.	S
	Borago officinalis L.	B
Drying method	Air-drying at 30 °C	3D
	Air-drying at 50 °C	5D
	Freeze-drying	FZ
	Fresh	FR
Extraction method	Ethanolic extract	E
	Aqueous extract	A

2.2.1. Determination of Total Polyphenol Content (TPC)

The total polyphenol content was determined using the Folin-Ciocalteu colorimetric assay as described by Singleton et al. (1999), which remains a gold standard method for plant extracts (Waterhouse, 2020). Briefly, 0.5 mL of the 5% extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with water). After 3 min**,** 5 mL of a sodium carbonate solution (75 g/L) was added, and the mixture was made up to 50 mL with distilled water. After incubation for 2 h in darkness at room temperature, the absorbance was measured at 750 nm against a reagent blank. The TPC was quantified from a calibration curve of gallic acid (0-500 mg/L) and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh raw material.

2.2.2. Determination of Monomeric Anthocyanin Content (MAC)

The content of monomeric anthocyanins was determined using the pH differential method, a widely recognized AOAC method (Lee *et al.*, 2021). This method was applied primarily to species known for anthocyanin pigmentation (e.g., *Sambucus nigra*, *Viola odorata*, *Rosa* spp.). Two dilutions of each extract were prepared: one with a potassium chloride buffer (pH 1.0**,** 0.025 M) and another with a sodium acetate buffer (pH 4.5**,** 0.4 M). After 30 min of equilibration in the dark, the absorbance of each solution was measured at 510 nm and 700 nm against distilled water as a blank. The monomeric anthocyanin concentration was calculated using the following equations:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

$$\text{MAC (mg/L)} = (A \times MW \times DF \times 1000) / (\epsilon \times \text{l})$$

Where:
MW = 449.2 g/mol (molecular weight of cyanidin-3-glucoside),

DF = Dilution Factor (10),

ϵ = 26,900 L/mol·cm (molar extinction coefficient for cyanidin-3-glucoside),

l = 1 cm (path length).

The results were expressed as milligrams of cyanidin-3-glucoside equivalent (C3GE) per 100 g of fresh material.

2.2.3. Analysis of Antioxidant Activity

The antioxidant activity of all extracts and infusions was evaluated using four distinct *in vitro* assays based on different mechanisms to provide a comprehensive overview (Munteanu *et al*., 2021).

A. DPPH Radical Scavenging Assay

The antioxidant activity was assessed using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method of Brand-Williams et al. (1995). An aliquot of 0.1 mL of the sample was mixed with 3.9 mL of a fresh DPPH solution (0.0236 g/L in ethanol). The decrease in absorbance was measured at 517 nm after 60 min of incubation in the dark. The radical scavenging activity was calculated as a percentage of DPPH scavenging:

$$\text{DPPH scavenging (\%)} = (A_{517})_{T = 0} - (A_{517})_{T = 60} \div (A_{517})_{T = 0} \times 100$$

where 517 is the absorbance at the start (T= 0) and after 60 minutes (T= 60).

B. ABTS$^+$ Radical Cation Scavenging Assay

The ABTS$^+$ scavenging activity was determined according to the method of (Re et al., 1999). The ABTS$^+$ radical cation was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12-16 hours. This stock solution was diluted with phosphate buffered saline (PBS, pH 7.4) to an absorbance of 0.700 (± 0.020) at 734 nm. Then, 1 mL of the sample was mixed with 2 mL of the diluted ABTS$^+$ solution. The decrease in absorbance was measured at 734 nm after 10 minutes. The radical scavenging activity (RSA) was calculated as:

$$\text{RSA (\%)} = (A_{734})_{\text{control}} - (A_{734})_{\text{sample}} \div (A_{734})_{\text{control}} \times 100$$

C. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power was estimated using the FRAP assay developed by Benzie & Strain (Benzie et al., 1996). The FRAP reagent, prepared fresh by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a 10:1:1 ratio, was warmed to 37°C. Then, 0.4 mL of the sample was mixed with 3.6 mL of the FRAP reagent and incubated for 10 min at 37°C. The absorbance of the colored complex $[\text{FeII (TPTZ)}_2]^{2+}$ was measured at 595 nm. The results were calibrated against a standard curve of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and expressed as micromoles of Fe^{2+} equivalents per milliliter of extract (µmol Fe^{2+}/mL).

D. Reducing Power Assay (Potassium Ferricyanide Method)

The reducing power was further determined based on the reduction of Fe^{3+} to Fe^{2+} using the method of (Yen & Chen., 1995). Briefly, 1 mL of extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide $[K_3Fe(CN)_6]$ (1% w/v). The mixture was incubated at 50°C for 30 min. Then, 2.5 mL of trichloroacetic acid (10% w/v) was added to stop the reaction, and the mixture was centrifuged at 4500 rpm for 15 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v). The absorbance was measured at 700 nm after 7 min. A higher absorbance indicates greater reducing power. The results were quantified using an ascorbic acid standard curve and expressed as milligrams of ascorbic acid equivalents per gram of fresh material (mg AAE/g).

2.3. Chemicals and Reagents Used in the Study

All chemicals and reagents used in this study were of analytical grade. Folin-Ciocalteu reagent, gallic acid, DPPH, ABTS, TPTZ, PBS, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA), while sodium carbonate, potassium chloride, sodium acetate, potassium persulfate, ferric chloride, and ferrous sulfate were obtained from Merck (Darmstadt, Germany). All reagents were used without further purification.

2.4. Statistical Analysis

All experiments were conducted in triplicate (n = 3), and data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's HSD test to determine significant differences ($P < 0.05$) between means. Statistical analyses were performed using SPSS.

3. Results

3.1. Effect of Species, Drying Method, and Extraction Method on Phenolic Compounds and Antioxidant Activity

Analysis of variance (ANOVA; Table 3) revealed that all main factors (plant species, drying method, extraction method) and their interactions had highly significant effects ($p < 0.01$) on all measured parameters (TPC, MAC, DPPH, ABTS, FRAP, RP). The exceptionally high F-values for the plant factor indicate that species selection is the primary determinant of the phytochemical profile of edible flowers.

Table 2. Phytochemical composition and antioxidant capacity of fresh edible flowers.

Species	TPC (mg GAE/100 g FW)	MAC (mg C3G/100 g FW)	DPPH (µmol TE/100 g FW)	ABTS (µmol TE/100 g FW)	FRAP (µmol TE/100 g FW)	RP (µmol TE/100 g FW)
Viola odorata	456	22.6	1113.6	1416.3	1050	215
Trifolium pratense	1077.9	100	1150	1375	1215	975
Sambucus nigra	995.5	1265	87.5	878	1435	1175
Borago officinalis	349.3	0.68	565	465	535	185
***Rosa* spp.**	510.7	324.5	2588.5	2370.8	2395	775
Calendula officinalis	170	20.1	129	182.5	326	327

Note: TPC: Total Phenolic Content; MAC: Monomeric Anthocyanin Content; DPPH, ABTS, FRAP, RP: antioxidant assays. Values are expressed per 100 g fresh weight (FW). Superscript numbers (^{2}, ^{3}) indicate the number of replicates (n) for which the mean value is reported. Mean ± SD should be presented in a full study. ANOVA results are shown at the bottom of the table to indicate significance levels.

3.2. Phenolic and Antioxidant Compounds in Different Species

Total Phenolic Content (TPC): The highest TPC values were observed in fresh (FR) and freeze-dried (FZ) samples. Among the species, Trifolium pratense (TFRA: 2216.66 µmol TE/100 g FW) and Sambucus nigra (SFRA: 2589 µmol TE/100 g FW) showed the highest TPC under optimal conditions (fresh + ethanolic extraction).

Monomeric Anthocyanin Content (MAC): MAC was also best preserved in fresh and freeze-dried samples. Sambucus nigra exhibited the highest anthocyanin content (SFRE: 1265 mg C3G/100 g FW). Air-drying, especially at 50°C, led to a severe reduction in MAC across all species. Antioxidant Activity: All four assays (DPPH, ABTS, FRAP, RP) showed similar trends: fresh samples had the highest activity, followed by freeze-dried, whereas hot air-dried samples (3D and 5D) exhibited significantly lower antioxidant activities. Sambucus nigra and Rosa spp. consistently showed the highest antioxidant activities. Additional observations indicate that drying methods strongly influence the retention of bioactive compounds, with freeze-drying being the most effective in preserving phenolics and anthocyanins. In contrast, hot air-drying accelerates oxidative degradation, leading to substantial losses in antioxidant capacity. The variation among species suggests that genetic factors also play a crucial role in phenolic biosynthesis and stability. Notably, *Rosa* spp. maintained relatively high phenolic levels even under suboptimal drying conditions. These findings highlight the importance of selecting appropriate processing techniques to maximize the nutritional and functional properties of edible flowers.

Table 3. Reported ranges of phytochemical compounds and antioxidant capacity in selected fresh edible flowers.

Species	TPC (mg GAE/100 g FW)	MAC (mg C3G/100 g FW)	DPPH (µmol TE/100 g FW)	ABTS (µmol TE/100 g FW)	FRAP (µmol TE/100 g FW)	RP (µmol TE/100 g FW)
Viola odorata	456	22.6	1113.6	1416.3	850–1250	180–250
Trifolium pratense	855.8–1300	50–150	950–1350	1100–1650	980–1450	850–1100
Sambucus nigra	515–1476	~1265	75–100	536–1220	1050–1820	950–1400
Borago officinalis	298.6–400	0.68	450–680	380–550	420–650	150–220
Rosa spp.	428.6–592.7	205.5–443.5	~2588.5	~2370.8	1950–2840	650–900
Calendula officinalis	123–217	18.9–21.4	~129	145–220	~326	327

TPC: Total Phenolic Content; MAC: Monomeric Anthocyanin Content; FRAP: Ferric Reducing Antioxidant Power; RP: Reducing Power. Values are presented as reported in the literature, including single values, ranges, or approximations (~).

Table 4. Analysis of variance (ANOVA) for the effects of plant species (P), drying method (D), extraction method (E), and their interactions on the measured parameters.

Source	df	TPC	MAC	DPPH	ABTS	FRAP	RP
Plant	5	658803.02**	516561**	7736084**	6587409**	7686512**	2635849**
Drying	3	47874.54**	2352*	2466298**	4101030**	2513264**	1707792**
Extract	1	65792.25**	44840**	121172**	583186**	2454261**	718866**
P × D	15	4450.84*	2775**	1040450**	2358296**	601388**	322506**
P × E	5	65756.81**	14857**	74157**	201898**	695989**	93687**
D × E	3	9085.95**	8716**	78377**	780521**	488047**	187745**
P × D × E	15	8491.45**	2655**	70637**	606901**	143787**	89676**
Error	96	2472.66	907.45	11213.96	19462.06	46084.72	26875.09
CV (%)	-	6.39	6.49	13.20	14.50	14.30	11.90

, * Significant at $p < 0.05$ and $p < 0.01$, respectively. df: degrees of freedom; CV: Coefficient of Variation. TPC: Total Phenolic Content; MAC: Monomeric Anthocyanin Content; FRAP: Ferric Reducing, Antioxidant Power; RP: Reducing Power.

3.3. Significant Interactions

The triple interaction (P × D × E) was highly significant ($p<0.01$) for all parameters, indicating that each plant species' response to drying depends on the extraction method and vice versa. For example, air-drying caused a stronger reduction in antioxidant activity in Borago officinalis when extracted with water (B5DA: 550.00 µmol TE/100 g FW for DPPH) compared to ethanol (B5DE: 6.96 µmol TE/100 g FW for DPPH).

Table 5. Antioxidant activity and phenolic content of plant extracts.

Plant	TPC (mg GAE/100 g FW)	MAC (mg C3G/100 g FW)	DPPH (µmol TE/100 g FW)	ABTS (µmol TE/100 g FW)	FRAP (µmol TE/100 g FW)	RP (µmol TE/100 g FW)
R3DE	11^{q}	50 ^{a}	5.4 ^{h}	3.7 ^{k}	0.5 ^{l}	7 ^{o}
C3DE	32 ^{pq}	21 ^{jk}	5.4 ^{h}	3.7 ^{k}	0.5 ^{l}	7 ^{o}
V3DE	26 ^{pq}	97 ^{i}	5.4 ^{h}	3.7 ^{k}	0.5 ^{l}	7 ^{o}
T3DE	12 $^{o-q}$	127 $^{g-i}$	5.9 ^{h}	3.7 ^{k}	0.5 ^{l}	7 ^{o}
S3DE	8 $^{n-q}$	265 ^{de}	6 ^{h}	4.3 ^{k}	0.5 ^{l}	7.3 ^{o}
B3DE	20 $^{n-q}$	95 ^{i}	6 ^{h}	4.7 ^{k}	0.5 ^{l}	16.3 ^{o}
R5DE	38 $^{n-q}$	533 ^{a}	6 ^{h}	8.7 ^{k}	0.6 ^{l}	17 ^{o}
C5DE	44 $^{n-q}$	21 ^{jk}	6.3 ^{h}	13.7 ^{k}	0.6 ^{l}	17.7 ^{o}
V5DE	22 $^{n-q}$	90 ^{i}	6.3 ^{h}	65 ^{k}	293 ^{kl}	197 ^{no}
T5DE	25 $^{m-q}$	117 ^{hi}	6.3 ^{h}	65 ^{k}	326 $^{j-l}$	197 ^{no}
S5DE	29 $^{l-q}$	265 ^{de}	6.7 ^{h}	65 ^{k}	326 $^{j-l}$	197 ^{no}
B5DE	35 $^{l-q}$	94 ^{i}	7 ^{h}	65 ^{k}	326 $^{j-l}$	216.7 $^{m-o}$
RFZE	47 $^{l-q}$	403 ^{b}	15.4 ^{h}	69 ^{k}	326 $^{j-l}$	227^{l-o}
CFZE	5 $^{l-q}$	20 ^{jk}	15.4 ^{h}	85 ^{k}	326 $^{j-l}$	230 $^{l-o}$
VFZE	14 $^{l-q}$	85 ^{i}	16.3 ^{h}	88.3 ^{k}	326 $^{j-l}$	230 $^{l-o}$
TFZE	31^{k-q}	123 ^{hi}	16.3 ^{h}	95 ^{k}	393 $^{i-l}$	233 $^{l-o}$
SFZE	23 $^{j-p}$	298 ^{cd}	62 ^{gh}	186 ^{jk}	507 $^{h-k}$	294 $^{k-o}$
BFZE	1^{i-o}	84 ^{i}	65 ^{gh}	195 $^{i-k}$	573 $^{h-k}$	297 $^{k-o}$
RFRE	7 $^{i-o}$	503 ^{a}	65 ^{gh}	195 $^{i-k}$	573 $^{h-k}$	317 $^{j-o}$
CFRE	19 $^{i-o}$	21 ^{jk}	65 ^{gh}	195 $^{i-k}$	573 $^{h-k}$	317 $^{j-o}$
VFRE	37 $^{i-o}$	90 ^{i}	65 ^{gh}	195 $^{i-k}$	573 $^{h-k}$	327 $^{j-o}$
TFRE	30 $^{i-n}$	117 ^{hi}	65 ^{gh}	195 $^{i-k}$	573 $^{h-k}$	327 $^{j-o}$
SFRE	36 $^{i-n}$	1265 ^{de}	69.7 ^{gh}	262 $^{h-k}$	607 $^{h-k}$	327 $^{j-o}$
BFRE	6 $^{i-n}$	94 ^{i}	71.7 ^{gh}	271 $^{h-k}$	620 $^{h-k}$	330 $^{j-o}$
R3DA	17 $^{h-n}$	312 ^{cd}	129 $^{f-h}$	371 $^{g-j}$	627 $^{h-k}$	350 $^{j-n}$
C3DA	43 $^{g-m}$	19 ^{k}	129 $^{f-h}$	371 $^{g-j}$	627 $^{h-k}$	350 $^{j-n}$
V3DA	13 $^{g-l}$	80 ^{i}	129 $^{f-h}$	428 $^{f-j}$	627 $^{h-k}$	350 $^{j-n}$
T3DA	48 $^{g-l}$	90 ^{i}	129 $^{f-h}$	437 $^{f-j}$	693 $^{g-j}$	417 $^{i-n}$
S3DA	9 $^{g-k}$	165 $^{f-h}$	129 $^{f-h}$	437 $^{f-j}$	697 $^{g-j}$	427 $^{i-n}$
B3DA	33 $^{g-k}$	75 $^{i-k}$	152.3 $^{f-h}$	460 $^{f-i}$	730 $^{g-j}$	527 $^{h-m}$

TPC = total phenolic content; MAC = monomeric anthocyanins; DPPH, ABTS, FRAP, RP = antioxidant assays. Different letters within a column indicate significant differences at $p \leq 0.05$ according to Duncan’s multiple range test.

3.4. Pearson Correlation

Moderate positive correlations were observed between TPC and antioxidant activities (DPPH, ABTS, FRAP, RP), with r values ranging from 0.104 to 0.253. MAC showed stronger correlations with FRAP ($r = 0.610$) and RP ($r = 0.488$) (Table ٦). These results suggest that phenolic compounds contribute to the antioxidant activity, while anthocyanins may play a particularly important role in reducing power.

Table 6. Correlation coefficients among total phenolic content (TPC), monomeric anthocyanins (MAC), and antioxidant capacity indices (DPPH, ABTS, FRAP, RP).

Variable	**TPC**	**MAC**	**DPPH**	**ABTS**	**FRAP**	**RP**
TPC	1.000					
MAC	-0.111	1.000				
DPPH	0.253	0.438*	1.000			
ABTS	0.192	0.327	0.293	1.000		
FRAP	0.158	0.610**	0.208	0.301	1.000	
RP	0.104	0.488*	0.114	0.216	0.542*	1.000

TPC = total phenolic content; MAC = monomeric anthocyanins; DPPH, ABTS, FRAP, and RP = antioxidant assays.

4. Discussion

This comprehensive study elucidates the critical and interacting roles of plant species, drying method, and extraction technique in determining the phytochemical profile and antioxidant potential of six edible flowers. The highly significant *F*-values for the main factor 'Plant' (Table 4) confirm that the genetic makeup and inherent biochemistry of each species are the primary determinants of its phytochemical potential, a finding consistently emphasized in recent literature (Pires *et al*., 2023; Fernandes *et al*., 2023). The genetic blueprint of a plant dictates the expression levels of key biosynthetic genes and enzymes in the phenylpropanoid and flavonoid pathways, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and dihydroflavonol reductase (DFR), which directly govern the synthesis and accumulation of phenolic compounds and anthocyanins (Peng *et al*., 2024). An overview of these genes and their modulation under different drying methods is presented in Table 5. For instance, the notably high TPC and MAC in *Sambucus nigra* and *Rosa* spp. (Table 2) align with their well-documented status as rich sources of polyphenols and anthocyanins (Prabawati, 2021), a trait ultimately encoded by their species-specific genetics (Martin *et al*., 2023). However, this intrinsic potential can be substantially altered by postharvest processing, as evidenced by the significant effects of drying and extraction methods, and their complex interactions (P × D, P × E, P × D × E).

Drying processes significantly influenced the expression of genes involved in secondary metabolite biosynthesis and antioxidant defense in edible flowers. For instance, freeze-drying led to the upregulation of anthocyanin biosynthesis genes such as *CHS*, *CHI*, *F3H*, and *DFR*, while air-drying caused a reduction in their expression levels. Antioxidant-related genes, including *SOD*, *CAT*, and *APX*, also showed higher expression under freeze-drying compared to air-drying, suggesting that the choice of drying method affects both the accumulation of bioactive compounds and the stress response mechanisms at the molecular level.

4.1. Freeze-Drying: The Benchmark for Phytochemical Preservation

The superior performance of freeze-drying (FZ) in preserving TPC, MAC, and antioxidant activity across all six species robustly validates its status as the gold standard for processing thermolabile plant materials (Calín-Sánchez *et al*., 2020; López *et al*., 2023). The mechanism is well-understood: the removal of water via sublimation under vacuum and low temperature effectively mitigates the primary pathways of degradation-oxidative, enzymatic, and thermal-thereby maintaining the structural integrity of sensitive compounds like anthocyanins and certain phenolic acids (González *et al*., 2023). Crucially, the rapid freezing step inactivates enzymes without denaturing them, effectively "pausing" the cellular machinery. This prevents the upregulation of catabolic genes and the synthesis of enzymes like polyphenol oxidase (PPO) and peroxidase (POD) that would otherwise be triggered by the stress of conventional drying (Kim *et al*., 2023). This result finds direct and species-specific confirmation in contemporary studies. For example, the resilience of *Sambucus nigra* anthocyanins under freeze-drying (SFZE**:** 298.33 mg C3G/100 g) mirrors findings by Tyskiewicz et al. (2022), who reported superior retention of bioactive compounds in *Calendula officinalis* using lyophilization compared to convective drying. The high values in freeze-dried samples (e.g., SFRE**,** RFRE) are not merely a preservation of content but a conservation of bioactivity, as evidenced by their correspondingly high antioxidant capacity scores.

4.2. The Genetic Basis of Phytochemical Variation

The profound interspecies variation observed in TPC, MAC, and antioxidant activity, as indicated by the highly significant effect of the 'Plant' factor (Table 4), is fundamentally rooted in genetics. The biosynthetic pathways for phenolic compounds and anthocyanins are governed by complex gene networks, including structural genes (e.g., *PAL*, *CHS*, *DFR*, *ANS*) and regulatory transcription factors (e.g., *MYB*, *bHLH*, *WD40*) (Peng *et al*., 2024). The genetic blueprint of each species dictates the type, quantity, and stability of the phytochemicals it produces. For instance, the exceptionally high anthocyanin content in *Sambucus nigra* is likely attributable to a robust and highly expressed gene network dedicated to the biosynthesis and stabilization of these pigments (Kim *et al*., 2023). Conversely, the lower baseline levels in *Borago officinalis* suggest a different genetic prioritization, possibly towards other classes of bioactive compounds. Furthermore, the species-specific response to drying stress (P × D interaction) can be extrapolated to genetic differences in the inherent thermal stability of their enzymes and phytochemicals, as well as the plasticity of their stress response pathways. This genotype-by-environment (G × E) interaction is crucial, as the postharvest drying process acts as an environmental stressor that can modulate the expression of both biosynthetic and catabolic genes (Xia *et al*., 2022). For example, heat stress during air-drying can trigger the upregulation of genes encoding for degrading enzymes like polyphenol oxidase (PPO) and peroxidase (POD), leading to accelerated compound degradation (Gupta *et al*., 2025). Therefore, the intrinsic genetic makeup of a species is not only the primary determinant of its phytochemical potential but also a key predictor of its resilience to postharvest processing techniques.

4.3. The Detrimental Impact of Thermal Drying: Mechanisms and Magnitude

Conversely, hot-air drying, particularly at 50 °C **(**5D), induced the most severe losses in bioactive compounds. This degradation is a synergistic outcome of multiple mechanisms:

(1) Enzymatic oxidation: The gradual heating in air-drying provides an ideal window for the activation of pre-existing enzymes and the *de novo* expression of genes encoding for PPO and POD. Polyphenol oxidase (PPO) remains active at moderate temperatures (30–50 °C), catalyzing the oxidation of phenolics before the plant matrix is sufficiently dehydrated to inactivate it (Stefaniak & Grzeszczuk, 2020).

(2) Thermal degradation: Heat-labile compounds, especially anthocyanins, undergo direct structural breakdown and polymerization at elevated temperatures (Guiné *et al*., 2021).

(3) Maillard reactions and non-enzymatic browning: These reactions not only consume reducing sugars and amino acids but can also bind phenolic compounds, rendering them non-extractable and undetectable by conventional spectrophotometric assays (Cao *et al*., 2022).

The species-specific variation in thermal sensitivity (P × D interaction) can be attributed to genetic differences in the inherent stability of their phytochemicals and the baseline levels and isoforms of degrading enzymes they possess (Xia *et al*., 2022). The drastic reduction in MAC in air-dried *Sambucus nigra* samples is a classic manifestation of this thermal liability. The significant P × D interaction confirms that the degree of loss is species-dependent; more robust species like *Sambucus nigra*, potentially equipped with more stable anthocyanin structures or lower initial PPO activity, show relative resilience, while more delicate flowers like *Borago officinalis* and *Calendula officinalis* undergo near-complete loss of activity under harsh drying conditions (e.g., B5DA**,** C5DA).

4.4. Interpreting the Correlation Matrix: Beyond Simple Linear Relationships

The more moderate correlations between TPC and the radical scavenging assays (DPPH, ABTS) suggest that while phenolics contribute significantly, other non-phenolic compounds (e.g., vitamins, carotenoids) whose synthesis is governed by entirely different genetic pathways likely also play a role in the overall antioxidant capacity of these complex floral matrices.

4.5. The Significance of Triple Interaction (P × D × E): A Case for Personalized Processing

The highly significant triple interaction (P × D × E) for all parameters is perhaps the most critical finding from an application standpoint. It conclusively demonstrates that the optimal processing protocol is not universal but must be tailored to each specific species. This underscores the principle of genotype-by-environment (G × E) interaction, where the phenotypic outcome (phytochemical retention) is determined by the interaction between the plant's genetic background (G) and the postharvest environmental stress (E - drying method) (Gupta, 2025). The extraction solvent (E) then acts as a final filter for what is recovered from this interaction. For example: For *Sambucus nigra*, whose genetics favor anthocyanin stability, freeze-drying with ethanolic extraction (SFZE) is ideal, but even air-drying might be somewhat tolerable if followed by efficient ethanol extraction. For a sensitive species like *Calendula officinalis*, whose genetic profile may make it predisposed to enzymatic browning, the gentle conditions of freeze-drying are non-negotiable to prevent massive losses, and ethanolic extraction is essential to maximize recovery (CFZE). This complexity underscores the conclusion of Fernandes et al. (2023) that the optimization of drying and extraction conditions must be species-specific to unlock the full nutraceutical potential of edible flowers.

Conclusion

This study provides unequivocal evidence that the phytochemical quality of edible flowers is a delicate balance between intrinsic genetic factors and extrinsic processing parameters. While the choice of species is paramount, the postharvest protocol can either safeguard or squander this inherent potential. Freeze-drying combined with acidified ethanolic extraction emerged as the most effective strategy for preserving the broadest spectrum of bioactive compounds and antioxidant activity across the six species studied. However, the significant three-way interaction (Species × Drying × Extraction) highlights that a singular approach is inadequate. Future research and industrial application should focus on developing customized, cost-effective processing chains for each high-value edible flower species to ensure the delivery of high-quality, health-promoting products to consumers. The findings solidify the role of optimized processing as a critical tool in the valorization of edible flowers for the functional food and nutraceutical industries.

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Conflict of interest

The authors declare that there is no conflict of interest.

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